

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph at page 1, line 3 of the application.

This application is a national stage application of ~~corresponds to~~ international patent application PCT/GB98/01619, filed June 3, 1998, and published as WO 98/55624 on December 10, 1998, which claims priority to 97113898.8 and 9811221.2, the disclosures of each which are is incorporated herein in ~~its~~ their entirety by reference.

Please replace the paragraph at page 26, line 40, with the following replacement paragraph:

Brief Description of the Drawings ~~Explanation of the Figures~~

Please replace the paragraph spanning page 26, lines 41, to page 27, line 6, with the following replacement paragraph:

Figures 1A-1E: Part A. Multiple sequence alignment of the predicted amino-acid sequences of RP-factor-like gene products from *M. luteus*, *M. tuberculosis*, *M. leprae* and *Streptomyces coelicolor*. Proteins similar to the RP-factor are derived from *M. tuberculosis* (accession nos. U38939, nt 2406-2765, and Z81368, nt 33932-34396) and *M. leprae* (accession nos. L01095, nt 12292-12759, and L04666, nt 25446-24921). The DNA sequences of interest in accession Z81 368 are also encompassed by accession AD000010. N-terminal residues corresponding to predicted Gram-positive signal sequences are underlined. The *M. leprae* L04666 sequence may also contain a short, 32 aa signal peptide.

At page 27, please replace the paragraph spanning lines 34-37, with the following replacement paragraph:

Figures 2A-and 2B: Part A. The sequence of the RP-factor-encoding gene and its predicted product. The nucleotide sequence is in lower case with PCR primers in bold. The predicted protein sequence is in upper case bold (single letter code). Protein and peptide microsequence data used for oligonucleotide design are in upper case italics.

Please replace the paragraph spanning page 27, line 41, to page 28, line 14, with the following replacement paragraph:

Figures 3A-3D: The elution profile of the resuscitation activity. Fractions eluted from the DEAE- sepharose column (see Materials and Methods) with 0.25 M KCl were applied to a Mono Q column which was developed with a 20ml linear gradient from 0.08 to 0.28 M KCl in 10 mM Tris-Cl buffer supplemented by 10% glycerol, pH 7.4. 10 ml of a diluted

suspension of starved cells (3.10^6 cells.ml⁻¹, total count $1.2.10^9$ cells.ml⁻¹) were added to 200 ml of LMM supplemented with 0.5 % w/v lactate and 0.05% yeast extract containing of 2 μ l of each fraction in 5-10 replicates in the Bioscreen instrument. For details see Materials and Methods. **A:** absorbance at 280nm and magnitude of KCl concentration. **B:** resuscitation activity. **C:** SDS-PAGE profile of the fractions following DEAE-cellulose and Mono Q chromatography. Lanes 1, markers (94,000, 67,000, 43,000, 30,000, 20,100, 14,400); 2, fraction from DEAE-cellulose column; 3, purified preparation (fraction number 8 from the Mono Q -column). **D:** Reduction of apparent lag phase of viable cells. 10 μ l of a diluted suspension of viable, stationary phase cells (viable count 20 cells) was added to 200 ml of LMM supplemented with 0.5 % w/v L-lactate and containing 2 μ l of each fraction (from a different experiment to that shown in parts A and B) in 5-10 replicates in the Bioscreen instrument. The apparent lag phase was estimated by extrapolating the exponential growth line to the abscissa.

At page 28, please replace the paragraph at line 15 with the following replacement paragraph:

Figures 4A-4B: Effect of purified RP-factor on *M. luteus*.

At page 28, please replace the paragraph spanning lines 31 and 32 with the following replacement paragraph:

Figures 5A-5C: Detection of RP-factor-like genes in *Micrococcus glutens*, *Mycobacterium smegmatis* and *Streptomyces rimosus*.

At page 28, please replace the paragraph spanning lines 6-14 with the following replacement paragraph.

Figures 6A and B : Effect of *M. luteus* RP-factor on the growth of *Mycobacterium smegmatis* (A) and *Mycobacterium bovis* (B) in batch culture as observed turbidimetrically. *M. smegmatis* was grown in broth E, to which was added RP-factor at 31 pMol/L. Cells were inoculated at a level of *circa* 200 per well, and growth was monitored in the Bioscreen instrument. *M. bovis* was grown in Sauton medium, as described in the Materials and Methods section, to which RP-factor (620 pMol/L) was either added or not. The inoculum was *circa* 1.10^5 cells.ml⁻¹, and the OD shown is the average of 10 separate determinations of 10 separate tubes.

At page 29, please replace the paragraph spanning lines 16-23 with the following replacement paragraph.

Figures 7A-7C: A: Purification of His-tagged RP-factor. RP-factor was expressed in *E. coli* HSM174(DE3) and purified as described *infra*. Shown is the SDS-PAGE profile of fractions following Ni^{2+} -chelation chromatography. The molecular weight (kDa) markers (SIGMA) were bovine serum albumin (67), ovalbumin (43), glyceraldehyde 3-phosphate dehydrogenase (36), carbonic anhydrase (30), soya bean trypsin inhibitor (20.1), and lactalbumin (14.4). Lane: 1, markers; 2, crude extract from *E. coli* containing pET19b vector; 3, crude extract from *E. coli* containing pRPF1; 4, purified recombinant RP- factor.

At page 29, please replace the paragraph spanning lines 36-42 with the following replacement paragraph.

Figures 8A and 8B: A: Anti-RP-factor serum inhibits the growth of *Micrococcus luteus*. Bacteria were inoculated at an initial density of 5×10^5 per ml into lactate minimal medium (LMM) and the $\text{OD}_{600\text{nm}}$ was monitored at intervals. Growth of the cultures was monitored over 140 hours at intervals. The samples labelled LMM + Ab and LMM + control Ab contain equivalent amounts of immune and pre-immune serum, respectively. Immune serum (Ab) and pre-immune serum (control Ab) were employed at a 1:1000 dilution.

At page 41, please replace the paragraph spanning lines 4-8 with the following replacement paragraph.

Figures 9A and 9B: Part A. Blocked alignment of nine RP-factors (as explained *infra*, MtubZ94752 may be a cognate receptor). Areas of sequence identity/similarity are indicated by the shaded areas. The *S. coelicolor* gene product shown is a fragment.